

## PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/21418>

Please be advised that this information was generated on 2017-12-05 and may be subject to change.

**TWO DISTINCT K<sup>+</sup>-ATPase ACTIVITIES IN RABBIT DISTAL COLON**S.L. Abrahamse, H.R. De Jonge\*, R.J.M. Bindels and C.H. Van Os<sup>1</sup>Department of Cell Physiology, University of Nijmegen,  
P.O. Box 9101, 6500 HB Nijmegen, The NetherlandsDepartment of Biochemistry I, Erasmus University of Rotterdam,  
P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

Received January 2, 1995

---

The distribution of K<sup>+</sup>-ATPase activity in surface and crypt cells from rabbit distal colon was studied. Separation of surface and crypt cells was validated using the multidrug resistance gene (*mdr-1*) product, P-glycoprotein, as marker for differentiated surface epithelial cells. Western blot analysis revealed a 6-fold higher expression level of P-glycoprotein in colonic surface cells. K<sup>+</sup>-stimulated ouabain-insensitive ATPase activity was present in surface and in crypt cells. In surface cells, this K<sup>+</sup>-ATPase activity was only partly inhibitable by 10  $\mu$ M SCH 28080, while in crypt cells K<sup>+</sup>-ATPase activity equalled SCH 28080-sensitive ATPase activity. These results strongly suggest the presence of two distinct K<sup>+</sup>-ATPases in colonic epithelial cells.

---

© 1995 Academic Press, Inc.

The mammalian colon is capable of active K<sup>+</sup> absorption and several transport characteristics implicate involvement of (H<sup>+</sup>-K<sup>+</sup>)-ATPase in this process [1-3]. Recently, a cDNA encoding the putative colonic (H<sup>+</sup>-K<sup>+</sup>)-ATPase has been isolated [4]. This new enzyme exhibited homology with gastric (H<sup>+</sup>-K<sup>+</sup>)-ATPase and with (Na<sup>+</sup>-K<sup>+</sup>)-ATPase. The localization of colonic (H<sup>+</sup>-K<sup>+</sup>)-ATPase within the epithelium is still a matter of controversy. In situ hybridisation identified the surface cells as the site of mRNA expression [5]. In contrast, a monoclonal antibody directed against the gastric (H<sup>+</sup>-K<sup>+</sup>)-ATPase was shown to recognize an epitope in the apical membrane of crypt cells [6].

Although (H<sup>+</sup>-K<sup>+</sup>)-ATPase mediates active K<sup>+</sup> absorption in the colon, its requirement in K<sup>+</sup> homeostasis has not been proven. Dietary K<sup>+</sup> restriction has been shown to increase K<sup>+</sup> absorption in rat distal colon and this could result from an increase in (H<sup>+</sup>-K<sup>+</sup>)-ATPase activity [7,8]. The aim of the present study was to determine the distribution of colonic (H<sup>+</sup>-K<sup>+</sup>)-ATPase in rabbit distal colon and to investigate the effect of dietary K<sup>+</sup> restriction on this enzyme activity.

---

<sup>1</sup>To whom correspondence and reprint requests should be addressed.

Abbreviations: PMSF, phenylmethylsulfonylfluoride; SCH28080, Schering compound 28080.



## **METHODS**

### **Isolation of apical membrane fractions**

For each experiment, colons were obtained from 2 New Zealand white rabbits of either sex weighing 2-3 kg. The animals had been fed with a standard chow for rodents (Hope Farms BV, Woerden, The Netherlands) and had free access to tap water. In K<sup>+</sup> restriction studies, rabbits were fed with a similar chow in which K<sup>+</sup> contents was lowered to 0.55% (w/w). Animals received a maximum of 150 g/rabbit/day for a period of 7 days, and had free access to distilled water. 24 h urine samples were collected, and K<sup>+</sup> concentration was determined flame photometrically (Eppendorf FCM6343, Germany). After 16 h starvation, the animals were killed by cervical dislocation, and subsequently bled. Dissected distal colons were stored in an ice cold solution containing 0.9% (w/v) NaCl and 1 mM HEPES/TRIS pH 7.4. Subsequently, colonic epithelial cells were separated into surface and crypt cells according to Gustin and Goodman [9] with a modification described by Wiener et al. [10]. Homogenization was performed in 50 mM sucrose, 1 mM EDTA, 0.1 mM PMSF, 5 mM HEPES/TRIS pH 7.4 for 1 min, using a polytron homogenizer. Homogenates were diluted with 3 volumes 250 mM sucrose, 1 mM EDTA, 0.1 mM PMSF, 5 mM HEPES/TRIS pH 7.4 and centrifugated for 10 min at 1000xg. Homogenization was repeated for the pellets, whereafter supernatants were pooled and the pellet collected. Supernatants were centrifugated at 100.000xg and from these crude membrane fraction (P100), enriched apical membranes (crypt cells C1, surface cells S2) were isolated as described by Abrahamse et al. [11]. Protein concentrations were determined using the Bio-Rad protein assay.

### **Gel electrophoresis, immunoblotting and autoradiography**

Polyacrylamide gelelectrophoresis (SDS-PAGE) was performed according to Laemmli [12] followed by immunoblotting and autoradiography as described by Van den Berghe et al. [13]. Samples (2 mg/ml in 30 mM TRIS/HCl, 1.5% (w/v) SDS, 5% (v/v) glycerol, 2.5% (v/v) 2-mercaptoethanol, 0.013% (w/v) bromophenol blue, pH 6.8) were heated for 5 min at 37 °C, prior to separation on 6% (w/v) polyacrylamide gels. For detection of Pgp, anti-Pgp antibody P-glycoCHECK C219 (Centicor, Belgium) was used at 1:500 dilution. Antibody binding was visualized using anti-mouse IgG antiserum and <sup>125</sup>I-labelled Protein A. Intensities were compared densitometrically, using a Gilford 2410S spectrophotometer (UK).

### **(H<sup>+</sup>-K<sup>+</sup>)-ATPase assay**

(H<sup>+</sup>-K<sup>+</sup>)-ATPase activity was measured at 37 °C in a total volume of 500 µl. Membrane fractions (5-20 µg) were preincubated with Triton X-100/digitonin 1:1 (w/w), 0.25 mg/mg protein, for 20 min on ice. Hereafter, samples were diluted into the assay mixture containing 0.1 mM EDTA, 1 mM ouabain, and 1 mM sodium azide in 30 mM imidazole/HCl pH 7.0. The reaction was initiated upon addition of 25 µl of 40 mM Tris-ATP, pH 7.0. K<sup>+</sup>-stimulated ATPase was measured as the difference in the rate of inorganic phosphate release in the presence of 5 mM KCl or 5 mM cholineCl. K<sup>+</sup>-stimulated SCH 28080-sensitive ATPase was determined as the difference in the rate of inorganic phosphate release in the absence or presence of 10 µM SCH 28080 in a 5 mM KCl containing medium. Reaction was stopped by addition of 0.5 ml 5% (w/v) trichloroacetic acid. Inorganic phosphate was determined as described by Mircheff and Wright [14].

### **Statistics**

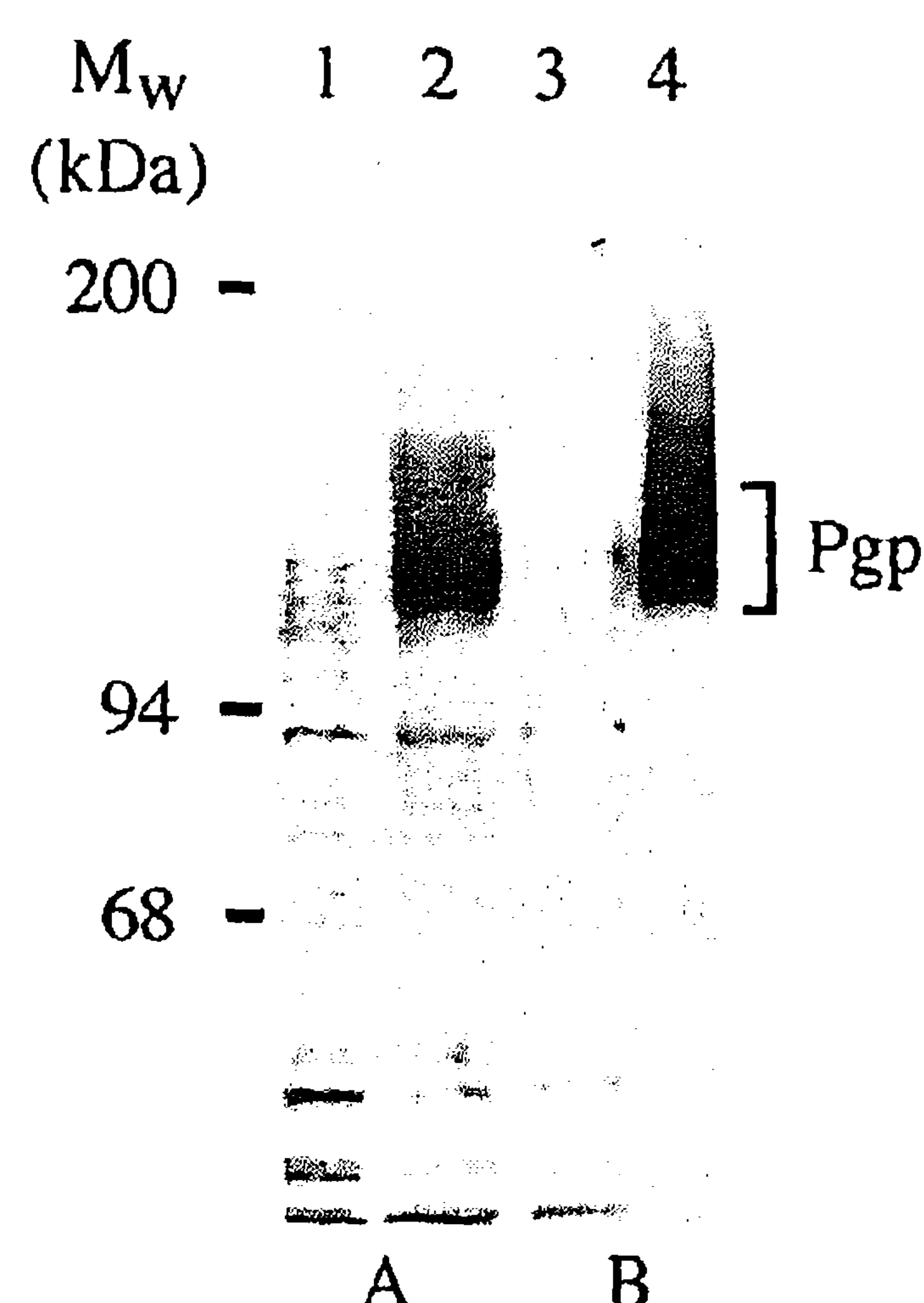
Results are reported as means ± standard errors (SE). Data were analyzed using StatView 512+ software (Abacus Concepts Inc., Berkeley, CA). When comparing two groups, an unpaired Student's t test was used. In case of more than two groups, analysis of variance was used. When overall effects were significantly different (P<0.05), experimental groups were compared by means of contrast analysis according to Fisher and Scheffé [15].

## **RESULTS**

### **Separation of surface and crypt cells**

The presence of the MDR gene (*mdr 1*) product in membrane fractions from surface and crypt cells was analyzed using Western blotting with a commercially obtained antibody against Pgp. As shown in Fig. 1, a protein band was detected in membranes from surface and crypt cells that was similar to Pgp with respect to molecular weight [16]. An increase in staining





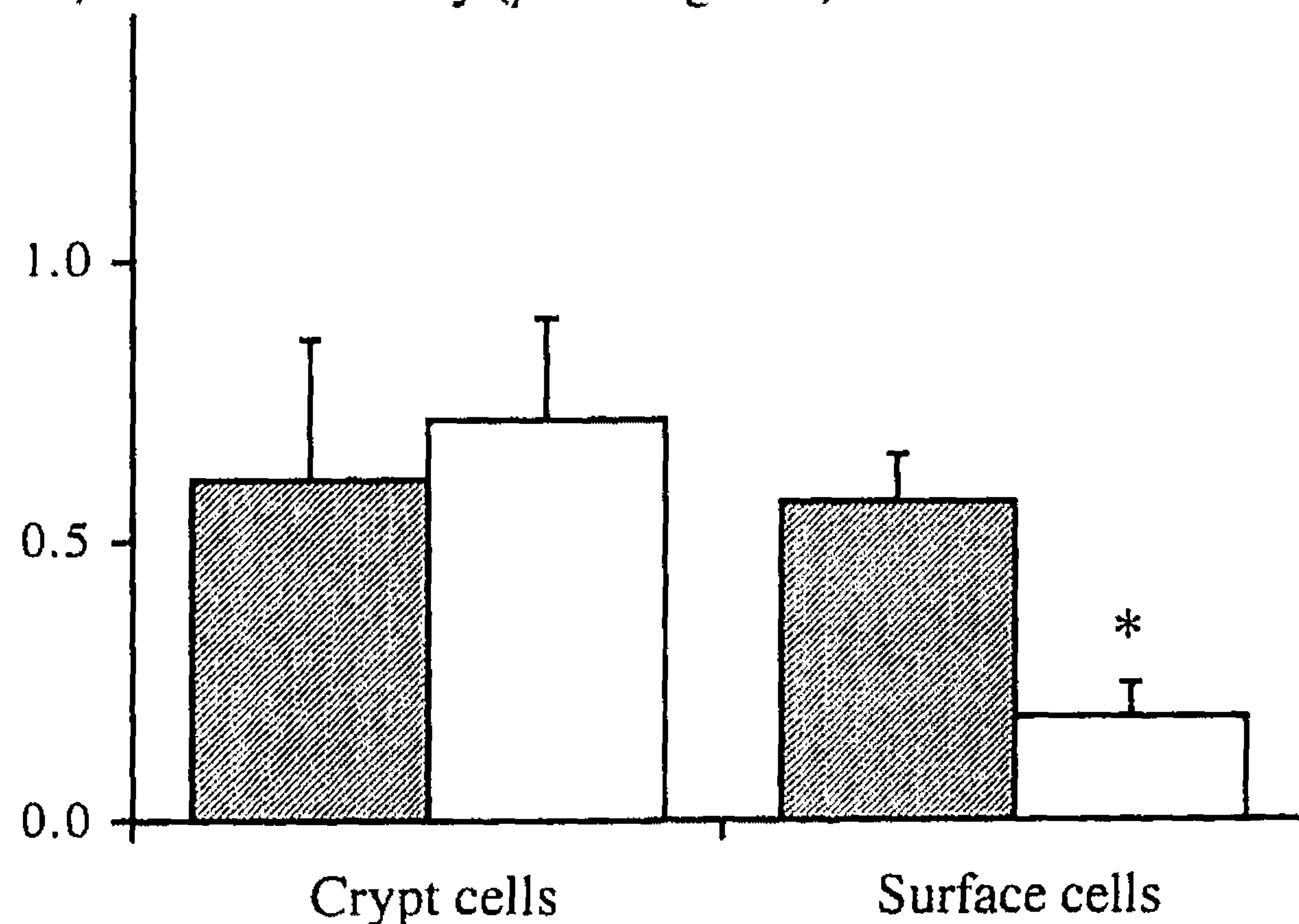
**Figure 1.** Binding of anti-P-glycoprotein antibody P-glycoCHECK C219 to proteins in membrane fractions from crypt (A) and surface cells (B) of rabbit distal colon. Lanes 1 and 3 contained 20  $\mu$ g microsome fraction (P(100)). Lanes 2 and 4 contained 20  $\mu$ g purified apical membrane fraction from crypt cells C(1) and from surface cells S(2), respectively. The positions of molecular weight markers (kDa) are indicated on the left.

intensity was observed between microsomal fractions and fractions enriched in apical membranes. Based on densitometric comparison of staining intensity, apical membranes from surface cells contained roughly 6 times more Pgp than apical membranes from crypt cells.

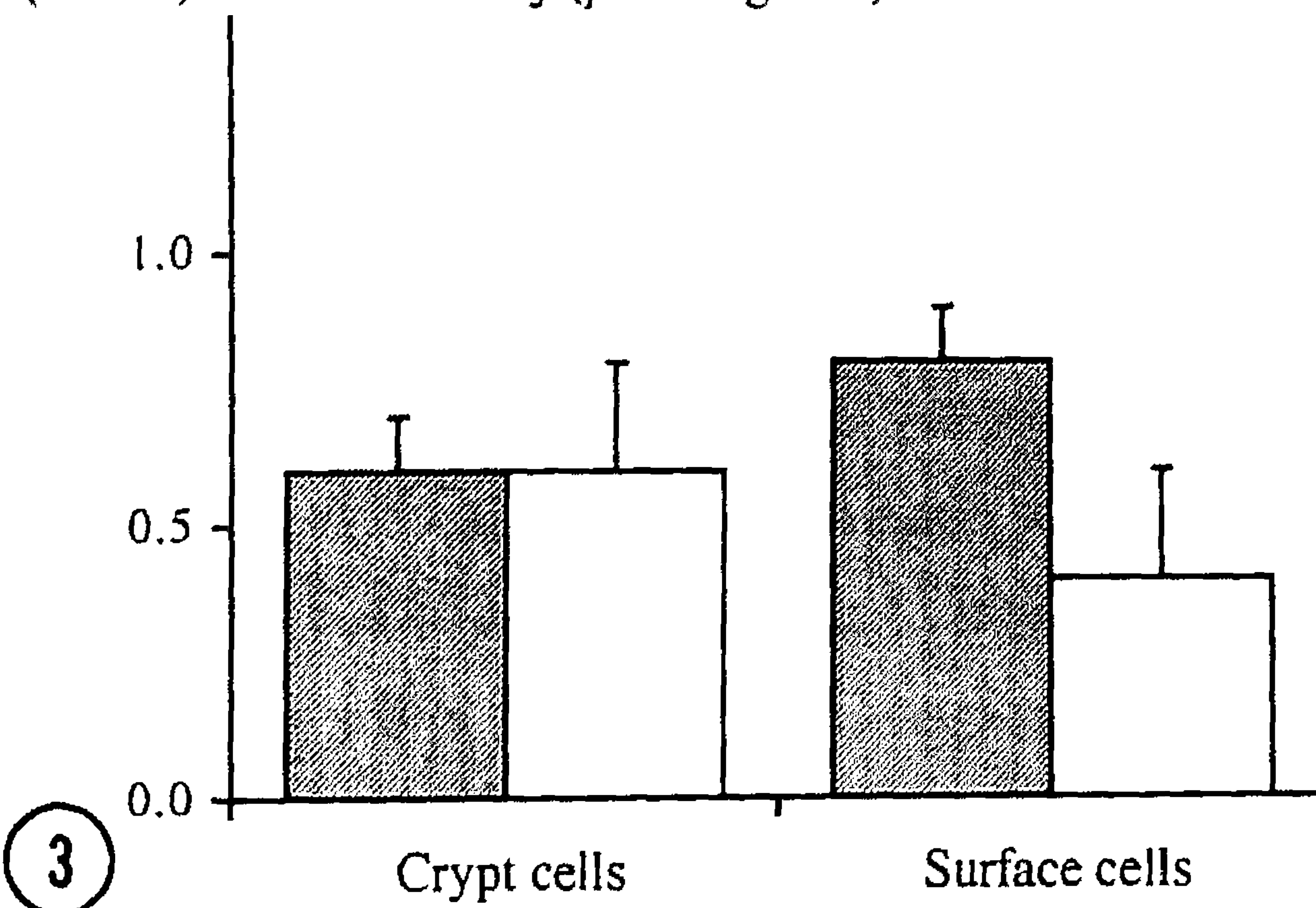
### **K<sup>+</sup>-ATPase activity in surface and crypt cells**

Colonic K<sup>+</sup>-ATPase activity was determined in the presence of ouabain, to inhibit (Na<sup>+</sup>-K<sup>+</sup>)-ATPase, and measured as K<sup>+</sup>-stimulated or K<sup>+</sup>-stimulated SCH 28080-sensitive ATPase activity [17] in homogenates from both surface and crypt cells. Fig. 2 shows the specific

(H<sup>+</sup>-K<sup>+</sup>)-ATPase activity ( $\mu$ mol $\cdot$ mg<sup>-1</sup> $\cdot$ h<sup>-1</sup>)



(H<sup>+</sup>-K<sup>+</sup>)-ATPase activity ( $\mu$ mol $\cdot$ mg<sup>-1</sup> $\cdot$ h<sup>-1</sup>)



**Figure 2.** (H<sup>+</sup>-K<sup>+</sup>)-ATPase activity in homogenates of surface and crypt cells from the distal colon of rabbits on a normal diet. (H<sup>+</sup>-K<sup>+</sup>)-ATPase activity was measured as K<sup>+</sup>-stimulated ATPase activity (hatched bars), or as K<sup>+</sup>-stimulated SCH 28080-sensitive ATPase activity (open bars), in the presence of 1 mM ouabain. Bars represent the means  $\pm$  SE of at least 3 experiments (\* Significantly different from K<sup>+</sup>-stimulated ATPase activity within the same cell fraction,  $P < 0.02$ ).

**Figure 3.** (H<sup>+</sup>-K<sup>+</sup>)-ATPase activity in homogenates of surface and crypt cells from the distal colon of K<sup>+</sup> restricted rabbits. (H<sup>+</sup>-K<sup>+</sup>)-ATPase activity was measured as K<sup>+</sup>-stimulated ATPase activity (hatched bars), or as K<sup>+</sup>-stimulated SCH 28080-sensitive ATPase activity (open bars), in the presence of 1 mM ouabain. Bars represent the means  $\pm$  SE of at least 4 experiments.



enzyme activities in these cell fractions. In crypt cells  $K^+$ -stimulated ATPase activity was equal to SCH 28080-sensitive ATPase activity. In surface cells, however, SCH 28080-sensitive ATPase activity was significantly smaller ( $P < 0.02$ ) than  $K^+$ -stimulated ATPase activity.

### **Effects of dietary $K^+$ restriction on $K^+$ -ATPase**

To find out whether dietary  $K^+$  restriction was of influence on colonic  $K^+$ -ATPase activity, rabbits were fed with a low  $K^+$  diet for 7 days. During this period renal  $K^+$  excretion significantly dropped from  $55 \pm 8$  to  $9 \pm 1$  mmol  $\cdot 24$  h $^{-1}$  ( $n=4$ ,  $P < 0.002$ ). Fig. 3 shows the  $K^+$ -ATPase activities as measured in homogenates from surface and crypt cells of rabbits on a low  $K^+$  diet.  $K^+$ -ATPase activities in both cell populations were not significantly different from activities in rabbits on a normal diet ( $P > 0.2$ , fig 2. compared with fig. 3).

### **DISCUSSION**

The colonic epithelium was separated into surface and crypt cells in order to determine the localization of  $K^+$ -ATPase activity. To show the effectiveness of the method applied for isolating surface and crypt cells, a marker for either cell fraction had to be used. The MDR gene (*mdr 1*), that confers multidrug resistance is known to be expressed in the human colon [18]. The gene product Pgp is known to function as a volume-regulated, ATP-dependent  $Cl^-$ -channel [19], and has been detected by immunohistochemical methods in the apical membrane of columnar epithelial cells [20]. Therefore, Pgp can serve as an additional marker for apical membranes of surface cells. In fact, an increase in staining on immunoblots was observed, when fractions from surface and crypt cells enriched in apical membranes were compared to a microsomal pellet (P100). A 6-fold higher expression was found in the apical membrane of surface cells when compared to crypt cells. An additional marker for surface cells is the amiloride sensitive  $Na^+$ -channel [21]. In another study we could detect amiloride-sensitive conductive  $^{22}Na^+$  uptake only in membrane vesicles derived from surface cells [11]. Therefore, we conclude that the method used in the present study effectively separates surface from crypt cells.

Surface and crypt cells contain similar activities of  $K^+$ -stimulated ATPase. Differences were only observed in SCH 28080-sensitive ATPase activity, which was 2-3 times higher in crypt cells than in surface cells. These results can be explained when we postulate that in the colon two distinct ( $H^+$ - $K^+$ )-ATPases are expressed, one with high sensitivity to SCH 28080 expressed in crypt cells, and a second not sensitive to SCH28080 expressed in surface cells. In other words, the colon contains a gastric-like ( $H^+$ - $K^+$ )-ATPase, sensitive to SCH 28080, that is most abundantly present in crypt cells. The second  $K^+$ -ATPase, insensitive to SCH 28080 and ouabain, must then be exclusively expressed in surface cells. The concept of two distinct  $K^+$ -ATPases in the distal colon offers an explanation for the discrepancy between immunohistological and in situ hybridization studies. A monoclonal antibody that recognizes an epitope of the gastric ( $H^+$ - $K^+$ )-ATPase have been shown to cross react with an epitope present in rabbit colonic crypt cells, whereas this epitope was absent, or at least undetectable in surface cells [6]. Like SCH 28080, this monoclonal antibody most likely reacts with a protein that is closely related to the gastric ( $H^+$ - $K^+$ )-ATPase. We can thus conclude that rabbit



colonic crypt cells contain a  $K^+$ -ATPase that is similar to the gastric ( $H^+$ - $K^+$ )-ATPase. Rabbit colonic surface cells contain a different  $K^+$ -ATPase that is not found in crypt cells. Interestingly, Crowson and Shull [4] characterized a cDNA encoding a protein with 63% amino acid identity to the  $\alpha$ -subunit of the gastric ( $H^+$ - $K^+$ )-ATPase and 63% amino acid identity to the  $\alpha$ -subunits of the ( $Na^+$ - $K^+$ )-ATPase. This novel protein was exclusively expressed in surface cells of rat distal colon [5]. This could well be the ouabain- and SCH 28080-insensitive  $K^+$ -stimulated ATPase.

Restriction in daily  $K^+$  intake has been described to increase active  $K^+$ -absorption in rat colon [7, 8], and ( $H^+$ - $K^+$ )-ATPase activity in rat kidney collecting tubule [22, 23]. The low  $K^+$  diet used in the present study was below the minimal  $K^+$  requirement for normal rabbit growth, and is known to increase  $K^+$  reabsorption in rabbit outer medullary collecting duct [24]. We observed that 24 h urinary  $K^+$  excretion was significantly reduced, but an increase in  $K^+$ -ATPase activity in homogenates of surface or crypt cells could not be measured. These results are in line with those of Tannen et al. [8] who demonstrated that in dietary  $K^+$  restriction the increase in  $K^+$  absorption in rat distal colon was due to a decrease in serosal-to-mucosal  $K^+$  transport, and not to an increase in mucosal-to-serosal flux. Thus, their and our results indicate that adaptation of ( $H^+$ - $K^+$ )-ATPase activity in  $K^+$  deficiency takes only place in the kidney.

Acknowledgments. The authors thank M.D. de Jong and G. J. Wildenbeest for technical assistance in part of this study. This investigation was supported by the Netherlands Organisation for Scientific Research (NWO grant no. 900-522-078).

## REFERENCES

- 1 Binder, H.J., and Sandle G.I. (1987) in: *Electrolyte Absorption and Secretion in the Mammalian Colon* (Johnson, L.R. ed) pp. 1389-1418, Raven Press, New York.
- 2 Smith, P.L., Sullivan, S.K., and McCabe, R.D. (1990) in: *Textbook of Secretory Diarrhea* (Lebenthal, E., and Duffey, M. eds) pp. 109-118, Raven Press, New York.
- 3 Suzuki, Y., Watanabe, T., and Kaneko, K. (1993) *Jap. J. Physiol.* 43, 291-298.
- 4 Crowson, M.S., and Shull, G.E. (1992) *J. Biol. Chem.* 267, 13740-13748.
- 5 Jaisser, F., Coutry, N., Farman, N., Binder, H.J., and Rossier B.C. (1993) *Am. J. Physiol.* 265, C1080-C1089.
- 6 Takeguchi, M., Asano, S., Tabuchi, Y., and Takeguchi, N. (1990) *Gastroenterology* 99, 1339-1346.
- 7 Foster, E.S., Sandle, G.I., Hayslett, J.P., and Binder, H.J. (1986) *Am. J. Physiol.* 251, G619-G626.
- 8 Tannen, R.L., Marino, R., and Dawson, D.C. (1986) *Am. J. Physiol.* 250, F483-F487.
- 9 Gustin, M.C., and Goodman, D.B.P. (1981) *J. Biol. Chem.* 256, 10651-10656.
- 10 Wiener, H., Turnheim, K., and Van Os, C.H. (1989) *J. Membrane Biol.* 110, 147-162.
- 11 Abrahamse, S.L., Bridges, R.J., De Jong, M.D., Bindels, R.J.M., and Van Os, C.H. (Submitted for publication).
- 12 Laemmli, U.K. (1970) *Nature* 227, 680-685.
- 13 Van den Berghe, N., Nieuwkoop, N.J., Vaandrager, A.B., and De Jonge, H.R. (1991) *Biochem. J.* 278, 565-571.
- 14 Mircheff, A.K., and Wright, E.M. (1976) *J. Membrane Biol.* 28, 309-333.
- 15 Snedecor, G.W., and Cochran, W.G. (1979) *Statistical Methods*, The Iowa State University Press, Ames.
- 16 Juranka, P.F., Zastawny, R.L., and Ling, V. (1989) *Faseb J.* 3, 2583-2592.

- 17 Wallmark, B., Briving, C., Fryklund, J., Munson, K., Jackson, R., Mendlein, J., Rabon, E., and Sachs, G. (1987) *J. Biol. Chem.* 262, 2077-2084.
- 18 Fojo, A.T., Ueda, K., Slamon, D.J., Poplack, D.G., Gottesman, M.M., and Pastan, I. (1987) *Proc. Natl. Acad. Sci. USA* 84, 265-269.
- 19 Valverde, M.A., Diaz, M., Sepúlveda, F.V., Gill, D.R., Hyde, S.C., and Higgins, C.F. (1992) *Nature* 355, 830-833.
- 20 Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M.M., Pastan, I., and M.C. Willingham, M.C. (1987) *Proc. Natl. Acad. Sci. USA* 84, 7735-7738.
- 21 Köckerling, A., Sorgenfrei, D., and Fromm, M. (1993) *Am. J. Physiol.* 264, C1285-C1293.
- 22 Cheval, L., Barletbas, C., Khadouri, C., Feraille, E., Marsy, S., and Doucet, A. (1991) *Am. J. Physiol.* 260, F800-F805.
- 23 Doucet, A., and Marsy, S. (1987) *Am. J. Physiol.* 253, F418-F423.
- 24 Wingo, C.S. (1987) *Am. J. Physiol.* 253, F1136-F1141.